Protocol - Growing Cells for Measurement

These are steps and questions related to growing up your cells to measure your finished devices.

Step 1: Streak out an agar plates with your organism containing the device and any control organisms you used *

Please check off each step that you followed. If you did anything differently or extra, please note that in "Other".

- ☐ Streak out 1 plate per device and control
- ☐ Incubate plates overnight (18-20 hrs or until colonies are clearly visible) at 37C
- ☐ Note the time grown in hours below in “Other”
- ☐ Other:

Please provide the positive control(s) you used. *

Note the organism, device, and/or plasmid used. We recommend BBa_I20270, a GFP expression device in the pSB1C3 backbone (chloramphenicol resistant).

Please provide the negative control(s) you used. *

Note the organism, device, and/or plasmid used. We recommend having one negative control where you test cells without any plasmid added and one negative control with an empty vector transformed (we recommend BBa_R0040, which is pTetR in pSB1C3).

What type of agar did you use for this step? *

For E. coli, we recommend LB Agar supplemented with the appropriate antibiotic. For antibiotic concentrations, please follow these guidelines: http://parts.igem.org/Help:Protocols/Antibiotic_Stocks

Step 2: Inoculate liquid culture with your experimental devices and controls. *

Please check off each culture that you setup. If you did anything differently or extra, please note that in "Other".

- ☐ Device 1: J23101+I13504
- ☐ Device 2: J23106+I13504
- ☐ Device 3: J23117+I13504

- [ ] Positive control (noted above)
- [ ] Negative control (noted above)
- [ ] Other:

What type of vessel or container did you use to grow your cells? *

- [ ] Test tube
- [ ] 15mL conical tube
- [ ] 96-well plate - round wells, flat bottom
- [ ] 96-well plate - round wells, round bottom
- [ ] 96-well plate - square wells, flat bottom
- [ ] 96-well plate - square wells, round bottom
- [ ] 96-deep well plate
- [ ] Flask
- [ ] Other:

Please provide any detailed information about your vessel / container below. *
For example: What dimensions were your test tubes? What volume was your conical tube or flask? If you used a flask, were they baffled or smooth?

If you used a test tube or conical tube, how were the tubes oriented in the incubator? *

- [ ] Upright
- [ ] At an angle
- [ ] Lying down
- [ ] N/A - used a different vessel
- [ ] Other:

What type of media did you use? *
For E. coli, we recommend Luria Broth supplemented with the appropriate antibiotic. For antibiotic concentrations, please follow these guidelines: http://parts.igem.org/Help:Protocols/Antibiotic_Stocks

What volume did you use to grow your cells? *
For test tubes, we recommend at least 3 mL of media. For 96-well plates, we recommend at least 150 uL of media.

Did you set up biological replicates in triplicate? *
Biological replicates are where different samples that are expected to be identical are measured. For example, if you are using E. coli, this would be done by measuring the fluorescence from three (3) different colonies containing the same device.

- ☐ Yes
- ☐ No

If you answered "No" to the previous question, state exactly how many biological replicates you ran for each construct.
Remember: one of the InterLab requirements is to measure your samples in biological triplicates.

Step 3: Incubate your liquid cultures. *
Please check off each step that you followed. If you did anything differently or extra, please note that in the "Other" box.

- ☐ Temperature at 37 C (if different, note in "Other" below)
- ☐ Shaking at 300 rpm (if different, note in "Other" below)
- ☐ Incubate for 16-18 hours (if different, note in "Other" below)
- ☐ Other:

Step 3: Continued

If you need more space to explain your growth conditions, please use the box below. We would expect this for mammalian work or non-traditional bacterial chassis - but all teams are welcome to add more details.

Plate Reader Measurement

This is a general guide for setting up your cells for plate readings. These steps and questions are meant to provide a general protocol and we ask that you follow them to the best of your ability. If you follow a different protocol, please note it in the final question in this section. If you did not use a plate reader, please answer the first question and then you may skip this rest of this section.

Did you measure your cells with a plate reader? *

- ☐ Yes
- ☐ No

Step 1: Obtain initial OD600 measurement of your overnight cultures.

Please check off each step that you followed. If you did anything differently or extra, please note that in "Other".

- ☐ Set your instrument to read OD600
- ☐ Setup a 96-well plate or cuvette with your cultures
- ☐ Take the measurement and record it
- ☐ Other:

Step 2: Dilute your samples to an OD600 of 0.5

Please check off each step that you followed. If you did anything differently or extra, please note that in the "Other" box.

- ☐ Calculate the dilution required for each sample
- ☐ Dilute each sample
- ☐ Re-measure your sample on OD600
- ☐ If your OD600 is within 5% of 0.5, proceed
- ☐ If your OD600 is outside that range, recalculate your dilution and remeasure until it's within 5%
- ☐ Other:

Step 3: Measure your samples

Please check off each step that you followed. If you did anything differently or extra, please note that in the "Other" box.

- ☐ Set your instrument to measure GFP
- ☐ Measure your cells
- ☐ Other:

Alternate Protocol or Additional Details

Did you follow a different protocol? Please describe your protocol below. You may also add more details below if you followed our protocol.

Other Forms of Measurement

Protocol for non-plate readers

Did you measure the cells with a different piece of equipment? Please describe your protocol below with as much detail as possible.